CONTACT-INHIBITED REVERTANT CELL LINES
ISOLATED FROM SV40-TRANSFORMED CELLS

IV. Microfilament Distribution and Cell Shape in
Untransformed, Transformed, and Revertant Balb/c 3T3 Cells

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ABSTRACT
A comparison is made of the ultrastructure of the cell periphery in three cloned cell lines: untransformed Balb/c 3T3 cells, SV40-transformed Balb/c 3T3 cells, and revertant cells obtained from the transformed cell line by a selection technique utilizing concanavalin A. Both thin-section and surface replication techniques are used for in situ examination of the cell lines. Microfilaments, 70 Å in diameter (called alpha filaments), are abundant in untransformed and revertant cell lines, particularly in the anterior expansions of the cells, which tend to have many microvilli and small pseudopodia. Alpha filaments are diminished in the anterior expansions of transformed cells, which contain large blunt pseudopodia and relatively few microvilli. Surface replicas confirm the impression gained from thin sections that transformed cells have a greater proportion of their cell surface involved in bulging pseudopodia than either untransformed or revertant cells. Since alpha filaments are shown to bind heavy meromyosin and are similar to F-actin, these filaments are thought to be important in cell motility. These observations suggest that a close relationship exists between decreased alpha filaments, bulging pseudopodia, and loss of contact inhibition of movement in transformed cells.

INTRODUCTION
Cultures of fibroblasts from normal tissue exhibit "contact inhibition" of growth, as defined by the fact that such cultures contain few dividing cells after confluence of the culture has been achieved (reviewed by 27, 43–45). In contrast, fibroblasts that have been transformed by an oncogenic virus, such as Simian virus 40 (SV40)1, lack contact inhibition of growth. A cloned transformed cell line is composed of a heterogeneous cell population since certain selection procedures can isolate a small number of contact-inhibited cells from cul-

1 Abbreviations used in this paper: ATP, adenosine triphosphate; Balb/c 3T3, cloned cell line of aneuploid fibroblasts derived from a Balb/c mouse embryo by Aaronson and Todaro (1); con A, concanavalin A; F-actin, fibrous actin; FUDR, fluorodeoxyuridine; HMM, heavy meromyosin; LMM, light meromyosin; MEM X 4, Eagle's Minimal Essentia Medium

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tures of these cell lines (34). The contact-inhibited variants of transformed cells are called revertant cells because they have regained the property of contact inhibition.

In two preceding papers (13, 28) some of the biochemical, growth, and ultrastructural characteristics of certain revertant cell lines were compared to those of transformed and untransformed cell lines. The revertant cell lines previously described were isolated from SV40-transformed Swiss 3T3 fibroblasts, using fluorodeoxyuridine (FUdR) as a selection agent according to the method of Pollack et al. (34). The results obtained with FUdR selected revertants were complicated by the ability of FUdR to act as a mutagen as well as a selection agent (12). This analogue causes a small degree of chromosomal pulverization as indicated by the presence of 4–6 minute chromosomes per revertant cell. Recently, Culp and Black (12) have attempted to circumvent the problem of chromosomal damage by utilizing the plant agglutinin concanavalin A (con A) as a selection agent. Since con A acts primarily at the cell surface, revertant cells are isolated by a pure selection process. This report describes some of the ultrastructural properties of untransformed, SV40-transformed, and con A selected revertant Balb/c 3T3 cells. Comparisons of the ultrastructure of these cells may further elucidate the mechanisms operative in maintaining contact inhibition. In this study, attention is focused on the cell periphery and its system of microfilaments since differences in microfilament distribution were previously found in the study of FUdR selected revertant cells when compared to the parental SV40-transformed cell line from which they were derived. An attempt is also made to relate changes in microfilament distribution to changes in the shape of cells as determined by surface replication techniques.

MATERIALS AND METHODS

Cell Lines

For this study, three cloned cell lines were examined. The contact-inhibited, nontransformed cells were from the A31 clone of Balb/c 3T3 cells. Transformation of this clonal line by SV40 virus resulted in the SVT2 cell line which lacked contact inhibition. These two cell lines were obtained from Dr. Stuart Aaronson (National Cancer Institute, National Institutes of Health) after 150 generations of growth. These cells were examined between their 5th and 15th passages in our laboratory. Two clones of revertant cells, clones 81 and 84, were selected by con A treatment of SVT2 cells, as previously described (12). The revertant cells were examined between their 5th and 15th passages. All cell lines were maintained in Eagle's MEM × 4 and were free of Mycoplasma contamination (12).

In addition to the Balb/c cell lines, cultures of untransformed Swiss 3T3 cells were used for certain experiments in which surface replication techniques were used. The origin, history, and maintenance conditions of this cell line have been previously described (13).

Electron Microscopy

Confluent and subconfluent cultures of Balb/c 3T3, SVT2, and con A revertant cells were prepared in situ for thin sections, as previously described (28). Cells were grown to the desired state of confluency in 72-96 h after subculturing on carbon-coated cover slips in glass Petri dishes containing MEM × 4 before processing for thin sections. Confluent cultures contained cells which were in contact with many neighboring cells as observed by light microscopy. In contrast, subconfluent cultures contained cells which were actively growing; both cells in contact with each other and isolated cells were present in these cultures.

Heavy meromyosin (HMM) was used as a test reagent for determining whether cytoplasmic microfilaments resemble fibrous actin (F-actin), according to the method of Ishikawa et al. (23) as modified by Pollard et al. (38). Cultures were grown just to confluence on carbon-coated cover slips and then glycinerized to 50% glycerol in phosphate buffer (0.1 M KCl, 0.005 M MgCl2, and 0.006 M Na2HPO4 at pH 7.1) which will hereafter be called standard phosphate buffer (SPB). The glycinerated cultures were stored for several days at 4°C. Purified chicken muscle myosin (6 mg/ml) was hydrolyzed by trypsin digestion (using 24 µg/ml TPCK-trypsin, Worthington Biochemical Corp., Freehold, N. J.) to produce HMM and light meromyosin (LMM). Soybean trypsin inhibitor was added to a final concentration of supplemented with a fourfold concentration of vitamins and amino acids, 10% fetal calf serum, and antibiotics; SPB, standard phosphate buffer (0.1 M KCl, 0.005 M MgCl2, and 0.006 M Na2HPO4 at pH 7.1); SV40, simian virus 40; SVT2, a cloned cell line derived after SV40 transformation of the A31 clone of Balb/c 3T3 cells; Swiss 3T3, an aneuploid fibroblastic cell line derived from a Swiss mouse embryo by Todaro and Green (49); U. S. P., United States Pharmacopoeia.
47 µg/ml in order to stop trypsin activity. The solution was dialyzed overnight at 4°C against 100 vol of 0.01 M sodium phosphate (pH 7.1) to precipitate LMM and unhydrolyzed myosin. The resultant suspension was centrifuged at 110,000 g for 90 min and the supernatant solution containing HMM was separated and dialyzed overnight against 5% glycerol in SPB. The final protein concentration of the HMM solution was 1.4 mg/ml. The cultures were rapidly deglycerinated to 5% glycerol-SPB and allowed to react with HMM in 5% glycerol-SPB for 16 h at 4°C. The cultures were washed several times with 5% glycerol-SPB over a period of 15 min. In control cultures, 5% glycerol-SPB was substituted for the HMM solution. The cells were fixed in 3% glutaraldehyde in 5% glycerol-SPB for 30 min at room temperature. The cultures were then processed in situ for thin sectioning as described (28).

Surface replicas were prepared of cultures grown for 72–96 h after subculturing on uncoated 2 x 2 mm fragments of glass cover slips in the bottom of Falcon plastic (Falcon Plastics, Div. of Biorquest, Oxnard, Calif.) or glass Petri dishes containing MEM x 4. Subconfluent or confluent cultures were fixed in glutaraldehyde and osmium tetroxide (28) and then air dried, using one of the following three methods. Some cultures were rinsed well in distilled water and air dried. Other cover slips were dehydrated in graded ethanol-water solutions and air dried in 100% ethanol. In the third method, cover slips were dehydrated in ethanol, rinsed in several changes of ethyl ether, and air dried at room temperature. The best preservation of total cell volume and shape was obtained by the third method using anhydrous ethyl ether, as would be expected from the low surface tension of ethyl ether (17 dyn/cm at 20°C) (24).

After drying, a platinum-carbon replica was cast of the surface of the cells on the cover slips, using the platinum-carbon electrode design of Moor (30). The replica was retrieved from the cover slip by digestion of the cells with Chlorox. The replica was washed in distilled water and examined either on uncoated 300-mesh grids or on Formvar-coated, 1 x 2 mm slot grids in a Siemens Elmiskop I electron microscope. The degree of structural distortion produced by the ether-air drying method was evaluated by comparison to results obtained from a single experiment on each cell line using the critical point-drying technique (8).

RESULTS

Light Microscopy

The morphology of the con A revertant cell lines, as determined by light microscopy, has been described and compared with that of the Balb/c 3T3 and SVT2 cell lines (12). Con A revertant cells are large, flat, polygonal cells which have an average of 90–100 chromosomes per cell. The parental SVT2 cell line is composed of small, rounded to spindle-shaped cells which have an average of 43 chromosomes per cell. The original untransformed Balb/c 3T3 cells are rather large, flat, polygonal cells. Thus the con A revertant and untransformed cells resemble each other in having a flattened polygonal morphology whereas the transformed SVT2 cells are smaller and have less tendency to spread onto the surface of the substrate (glass, carbon, or plastic).

Electron Microscopy

In thin sections of the untransformed Balb/c 3T3 cells, the cytoplasm subjacent to the plasma membrane contains a population of fine filaments, often called "microfilaments" (e.g., 51). In a previous study (28), we divided the population of microfilaments into two groups: alpha filaments and beta filaments. Alpha filaments are 60-80 Å in diameter, and tend to be aggregated into felt-like meshworks in which the filaments are often parallel to one another (Fig. 1). The length of individual alpha filaments is difficult to determine due to the close association of the filaments with one another and the consequent superimposition of many filament images within the section thickness. Alpha filaments extend into the fine filopodia and microvillous extensions of the cell surface. Focal regions of dense staining are found in the aggregates of alpha filaments and also where these filaments contact the cytoplasmic surface of the cell membrane. Beta filaments are approximately 100 Å in diameter and tend to course in loose fascicles in the cytoplasm (illustrated in 28). Individual beta filaments often can be followed for several microns in the plane of a single thin section. Beta filaments are primarily in the endoplasmic region of the cell.

Cells in culture are often polarized in the direction of movement. When a normal fibroblast is spread onto the surface of a cover slip and photographed by time-lapse cinephotomicrography, Abercrombie et al. (2-6) found that the portion of the cell which is most anterior in the direction of movement is usually spread into a thin, broad, flat region termed the anterior expansion. The sides of the cell are often straight or slightly curved. The posterior or trailing region of the cell is commonly tapered and drawn out at the tip into many fine fingerlike processes which remain
Figure 1 Electron micrograph at intermediate magnification of a thin section through the anterior portion of an untransformed Balb/c 3T3 cell in a subconfluent region of culture. A closely meshed, filamentous network is formed by "alpha" filaments (A filaments) which are 60-80 Å in diameter and often are parallel to each other (at arrows). Focal regions of dense staining (DR) are present in the network.

× 22,000.
attached to the surface of the cover slip. These long cytoplasmic processes have clublike ends where they attach to the cover slip and are called retraction fibers (see 47). In thin sections cut parallel to the surface of the cover slip, anterior expansions of cells can be identified in subconfluent regions of culture. In highly confluent cultures, anterior expansions are not evident in contact-inhibited cells but can be found in transformed cells.

Alpha filaments are unevenly distributed within the peripheral cytoplasm of the cell. In subconfluent and confluent cultures, alpha filaments are abundant in untransformed Balb/c 3T3 cells (Fig. 1) and in the con A revertant cells (Fig. 2). In subconfluent regions of the cultures, the alpha

![Figure 2](image)

Figure 2 A portion of a thin section through the anterior region of a con A revertant 3T3 cell in a subconfluent region of culture. Sheets of alpha filaments (A filaments) are prominent and have focal regions of dense staining (DR). × 31,200.

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filaments present in the anterior expansions of these two types of cells are commonly parallel to one another but also may be dispersed into a meshwork containing filaments oriented in different directions. Regions of anterior expansions may have few alpha filaments but such regions are a minor proportion of the anterior expansions of untransformed Balb/c 3T3 cells and con A revertant cells. Along the sides and near the trailing portion of the cell, the alpha filaments are particularly compact and parallel. Alpha filaments are most prominent in untransformed and con A revertant cells in cultures that are fixed very near the time when confluency is achieved. As also noted in our previous study (28), alpha filaments are abundant near regions of cell-to-cell attachment particularly at adherens junctions (Fig. 3) (for terminology, see 15, 29). Adherens junctions are prominent in confluent cultures of untransformed and revertant cells (Fig. 3). At an adherens junction, the apposed plasma membranes of adjacent cells are separated by a 200 Å wide cleft of extracellular space. Also, there is a condensation of dense fibrillar material at the cytoplasmic surfaces of the membranes.

In contrast, a typical SVT2-transformed cell is small and tends to have a relatively small anterior expansion. In thin sections, the SVT2 anterior expansions frequently lack parallel sheets and bundles of alpha filaments (Fig. 4). However, along the sides and near the trailing region of SVT2 cells, compact layers of alpha filaments may be present but are not as prominent as in untransformed and revertant cells. Beta filaments and microtubules are also uncommon in the anterior expansions of SVT2 cells. There is some individual variation in the degree of alteration of alpha filament distribution among the cells in an SVT2 culture. Most of the transformed cells show a striking decrease in the abundance of alpha filament sheets near the anterior expansion when compared to untransformed cells. (Compare Fig. 4 with Figs. 1 and 2.) Adherens junctions are less prominent in confluent cultures of SVT2 cells when compared to confluent cultures of untransformed Balb/c or con A revertant cells.

The con A revertant cell lines (clones 81 and 84; see 12) closely resemble each other. The ectoplasmic region is flattened into a large anterior expansion which contains abundant alpha filaments (Fig. 2) as well as beta filaments and microtubules.

In order to determine whether alpha filaments are actinlike, HMM-binding studies have been performed using untransformed Balb/c 3T3 cells in cultures that have just become confluent (Fig. 5). In control cells glycerinated to 50%, deglycerinated to 5%, and fixed with glutaraldehyde, the alpha filaments tend to be more tightly packed in bundles (Fig. 6) than those in unglycerinated control cells (Fig. 5). The alpha filaments predominately appear as linear strands whose surfaces are smooth in the untreated control and glycerinated control cells. After exposure to HMM, the bundles of alpha filaments are less compact and the individual filaments have spiked surfaces (Figs. 7 and 8). This spiked appearance is similar to the images that others have called "arrowhead complexes" which are formed by the interaction of HMM with F-actin. No HMM binding to beta filaments was observed in our cultured cells. This selectivity of HMM binding was also observed by Ishikawa et al. (23).

After it was demonstrated that alpha filament sheets are less abundant in transformed cells than in untransformed and revertant cells, surface replication experiments were performed to determine whether this decrease in sheets of alpha filaments in transformed cells is correlated with an alteration in cell shape. In preparing specimens for replication, different methods of specimen drying were useful in delineating various aspects of cell morphology. The ether-air drying method for specimen preparation gave results comparable to those obtained with critical point drying. With the critical point method, cellular collapse was not observed, but with ether-air drying, the meniscus of the anhydrous ethyl ether causes some collapse of cellular volume particularly near the nucleus. The collapse at the cell perimeter is minimal since the positions of upright microvilli are preserved. In preparations dried in ethyl alcohol, or United States Pharmacopoeia (U. S. P.) grade ethyl ether, greater collapse of cell volume occurs and the microvilli tend to lose their upright positions. Such partial collapse is useful for localization of the nucleus, lipid droplets, and mitochondria. When cells are dried in distilled water, the collapse is extreme but the images produced are useful for determining the location of some cytoplasmic structures smaller than mitochondria. The cellular collapse causes loss of height above the surface of the cover slip rather than a change in shape of the cell perimeter.

Surface replicas show that the peripheries of untransformed Balb/c 3T3 cells and Swiss 3T3
FIGURE 3 A region of cell-to-cell contact in a confluent culture of untransformed Balb/c 3T3 cells. Alpha filaments (at single arrows) are abundant near cell processes that are attached by adherens junctions (at double arrows). Regions of extracellular space (ECS) separate the two cells. × 34,500.
FIGURE 4 Thin section showing the anterior region of one SVT2 cell and the edge of the posterior portion of an adjacent SVT2 cell in a subconfluent region of the culture. Frequently, the anterior expansions of SVT2 cells form broad pseudopodia (P) containing few membranous organelles. Ribosomes (Rib) are abundant and a few profiles of rough endoplasmic reticulum (Rer) are present. Alpha filament sheets are rarely seen in such anterior protrusions. In the posterior region, bundles of alpha filaments (at arrow) are adjacent to the plasma membrane. \( \times 28,300. \)
cells have a mixture of two types of protrusions from the anterior expansion (Fig. 9). One type of protrusion is a sheet of cytoplasm with a slightly irregular edge (Fig. 9). These regions are pseudopodia, frequently ca. 0.5 μm in width. The second type of protrusion from the anterior expansion is a sheet of cytoplasm with prominent microvilli projecting from the edge (Fig. 10). These microvilli often are approximately 0.1 μm in diameter and 0.5-2 μm in length. In preparations dried in distilled water, ridges continue into the cytoplasm from the base of each microvillus (Fig. 10). When fixed and dried in distilled water, the anterior expansion becomes quite flattened so that fine ridges are evident in the cytoplasm underlying the membrane (Fig. 11). These ridges correspond in size and location to cytoplasmic bundles of microfilaments and possibly microtubules as well.

The con A revertant cells usually have very thin, large anterior expansions with prominent microvilli at the edges (Fig. 12). In contrast, the transformed SVT2 cells tend to be spindle shaped with one or two small expansions at the anterior edge of the cell (Fig. 13). When most of the SVT2 cells in a culture have spread onto the surface of the cover slip, the anterior expansions predominantly have the shape of pseudopodia that may be 4-15 μm in width and 1-2 μm long. Such a relatively large proportion of the cell surface devoted to pseudopodia is rare in untransformed and revertant cells. These SVT2 pseudopodia have few well-formed microvilli. An occasional SVT2 cell has more microvilli than are found on the majority of the SVT2 cells, but the interpretation of this incidental observation is unclear. Retraction fibers can commonly be seen at the trailing region of the transformed cell.

**DISCUSSION**

This study demonstrates an abnormality in transformed cells seen in thin sections that is correlated with an abnormality seen in surface replicas. In thin sections, few bundles and sheets of alpha filaments are found in the anterior expansions of the transformed cells. Also, thin sections suggest that the regions with few sheets of alpha filaments in transformed cells represent broad protrusions from the cell surface. In surface replicas, transformed cells often have relatively large pseudopodia with few microvilli whereas untransformed and revertant cells have many microvilli and only small pseudopodia. In collapsed anterior expansions of untransformed cells, surface replicas demonstrate long ridges with the dimensions of bundles of alpha filaments. Such ridges are not prominent in the collapsed expansions of transformed cells.

We have previously shown that transformed cells have fewer alpha filament sheets than untransformed and FUdR revertant cells (28). On the basis of that study, the diminution of alpha filament sheets could not be definitely related to pseudopodial formation since anterior expansions of the cells were not reconstructed from serial sections. In the present study, there is a correlation between large pseudopodia on transformed cells seen in surface replicas and a diminution of alpha filament sheets in thin sections. In addition, this study extends our previous observations (28) to another cell line and to another type of selection process for the isolation of revertant cells. The fact that con A selected revertant cells have sheets of alpha filaments further supports a correlation between the presence of alpha filament sheets and contact inhibition. Trinkaus and Lentz (50) observed that embryonic cells showing contact inhibition of movement have areas of cell-to-cell contact with abundant cortical filaments. In contrast, embryonic cells lacking contact inhibition have broad extensions of cytoplasm containing few membranous organelles (and apparently few cortical filaments as well, e.g., Fig. 13 of reference 50).
Figure 9  Surface replica showing an anterior expansion of an untransformed Balb/c 3T3 cell in subconfluent culture. Two types of protrusions are present: small pseudopodia (P) and protrusions with fine microvilli (M) at the edge. Due to slight collapse of the cell during drying by the U. S. P. ether-air method, a bulge is apparently at the position of a mitochondrion (at arrow) X 10,600.

Of course, a correlation between two events does not necessarily indicate a cause and effect relationship between them. For example, it is possible that the condensation of sheets of alpha filaments and contact inhibition of movement are both secondary to a change at the cell surface when adjacent cells come into contact.

An important role for alpha filaments in cell motility has been suggested by many studies (e.g., 7, 11); of particular interest are those of the fine structure of the motile slime mold Physarum polycephalum (20, 31, 32, 39). Rhea (39) has observed that, at the advancing ends of cytoplasm of Physarum, a "hyaline" region of cytoplasm forms...
the most anterior portion. This hyaline cytoplasm appears free of bundles of filaments and most membranous organelles. In contrast, more posterior regions of the *Physarum* have prominent sheets of cortical filaments, similar to alpha filaments. These observations correlate with our observation that alpha filament sheets are not prominent in the large pseudopodia of transformed cells but, when present, are found principally along the sides and near the posterior region or “tail” of the cell. Also, Griffin (20) found that sessile *Physarum* have prominent cortical filaments and that motile *Physarum* have fewer cortical filaments. Griffin’s observations are similar to our findings that confluent, relatively sessile, untransformed 3T3 cells have more abundant sheets of alpha filaments than the confluent, motile, transformed cells.

Our studies utilizing HMM binding demonstrate that alpha filaments are similar to F-actin. The validity of using HMM as a test reagent for the presence of F-actin in thin sectioned cells has been determined by the previous work of Ishikawa et al. (23) and Pollard et al. (36, 37). When 3T3 fibroblasts are incubated with HMM, the alpha filaments bind HMM to give a spiked appearance to the filaments. Others have described similar spiked filaments as showing arrowhead complexes in thin sections of nonmuscle cells (23, 38). The demonstration that alpha filaments are actin-like strengthens the possibility that alpha filaments are...
part of a contractile system that is important in cell motility. Such a possibility is supported by the finding of Hoffmann-Berling (21, 22) that glycerinated fibroblasts contract on exposure to adenosine triphosphate (ATP), a property commonly shown by the actomyosin system of muscle cells (see also 26). Filaments, similar to alpha filaments, have been shown to be important in primitive motile systems. In Physarum, the cortical filaments bind HMM and are similar to F-actin (31, 32). In Amoeba proteus and Acanthamoeba castellanii, Pollard and co-workers (36, 37, 38) have demonstrated the presence of 70 Å actinlike filaments that bind HMM and appear in motile cytoplasmic extracts (35).

A possible relationship between alpha filaments and either cell-to-cell or cell-to-substrate attachment sites must be considered in an analysis of the difference in alpha filament distribution between transformed cells and untransformed and revertant cells. In this and our previous study (28), alpha filaments were noted to be intimately associated with regions of cell-to-cell attachment. Abercrombie et al. (7) found that cortical filaments (similar to alpha filaments) insert on the plasma membranes via dense plaques at sites of cell-to-substrate attachment. Although many of the details of cell motility in culture remain to be elucidated, specialized cell attachment sites are probably one important component of the cellular

Figure 11  Surface replica of a large, thin anterior expansion (AE) on an untransformed Swiss 3T3 cell. This preparation was dried in distilled water. The extreme cellular collapse during drying allows localization of bundles of cytoplasmic filaments (and perhaps microtubules) by causing them to form ridges (at arrows) in the surface of the cell. A few small pseudopodia (P) and a microvillus (M) are present at the leading edge of this expansion. × 7500.
motile apparatus. Certain aspects of the contact-inhibition phenomenon may be related to the size and number of attachment sites formed by cells in culture (4, 19, 49).

In this study, transformed cells have a greater proportion of the cell surface devoted to pseudopodia than either untransformed or revertant cells. Previous studies by other workers indicate that the formation of large pseudopodia may be an important characteristic of malignant cells even though pseudopodia are not restricted to malignant cells. Abnormal bulging pseudopodia have been observed in vitro on human colon cancer cells (17) and on astrocytes that have been transformed by Rous sarcoma virus (25), but neither of these studies related the surface protrusions to the distribution of alpha filaments. In vivo, pseudopodial protrusions from the cancer cell surface have been observed in squamous cell carcinomas (9, 18, 42, 46) and adenocarcinomas (14). These pseudopodia have often been considered as the earliest indication of malignant invasion (9, 46). The pseudopodia from cancer cells in vivo usually contain relatively few membranous organelles and few
Figure 13 Surface replica of an anterior expansion of an SVT2-transformed cell that lacks microvilli. In this example, the entire anterior expansion probably represents a large pseudopodium. Such large pseudopodia are rare in untransformed and revertant cells. Cytoplasmic collapse during U. S. P. ether-air drying has not shown ridges ordinarily produced by bundles of cytoplasmic filaments. X 10,000.
100 Å tonofilaments (18, 46). No comments have been made concerning the distribution of 70 Å microfilaments in cancer cells in vivo except in the case of smooth muscle tumors. Ferenczy et al. (16) showed a marked decrease in the prominence of actomyosin filaments in malignant smooth muscle cells when compared to cells forming benign tumors of smooth muscle. The fact that pseudopodia appear early in carcinogenesis also indicates that the cause of pseudopodial formation may be important. Stoker (44) has reported that multiple pseudopodia appear on cells within 18 h after infection with a transforming virus. Pseudopodia also appear early in chemical carcinogenesis (18, 42). However, pseudopod formation is not restricted to malignancy since other studies show that regenerating stratified squamous epithelium also have large pseudopodia (33, 40, 41). Both regenerating epithelium and malignant epithelium are similar in that they have lost contact inhibition of movement and growth, temporarily for regenerating cells and permanently for most cancer cells.

Since it seems likely that a contractile system, in part composed of alpha filaments, regulates cell motility, decreased alpha filament sheets may be important in the loss of contact inhibition of movement in transformed cells. It is conceivable that alpha filaments play a role in contact inhibition of movement since dense aggregates of alpha filaments are present at regions of cell-to-cell contact in those cell lines showing contact inhibition. Condensation and contraction of alpha filaments after cell contact may increase the viscosity of the ectoplasm and produce an immobilization of the cell in the region of contact. There is a close correlation between contact inhibition of movement and contact inhibition of growth that has been observed in a variety of experimental conditions (2–6, 10, 19). It is tempting to speculate that immobilization of the cell surface may be a factor in the regulation of growth. Thus, it is possible that the regulation of alpha filaments is important in the complex regulation of cell movement and growth.

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